

THE GROWTH AND HARVESTING OF ALGAE IN A MICRO-GRAVITY ENVIRONMENT

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ABSTRACT

Algae growth in a micro-gravity environment is an important factor in supporting man's permanent presence in space. Algae can be used to produce food, oxygen, and pure water in a manned space station. A space station is one example of a situation where a Controlled Ecological Life Support System (CELSS) is imperative. In setting up a CELSS with an engineering approach at the Aerospace department of the University of Colorado, questions concerning algae growth in micro-g have arisen. The Get Away Special (GAS) Fluids Management project is a means through which many questions about the effects of a micro-g environment on the adequacy of growth rates, the viability of micro-organisms, and separation of gasses and solids for harvesting purposes can be answered. In order to be compatible with the GAS tests, the algae must satisfy the following criteria: 1) rapid growth rates 2) sustain viability over long periods of non-growth storage 3) very brief latency from storage to rapid growth. Testing indicates that the overall growth characteristics of *Anacystis Nidulans* satisfy the specifications of GAS's design constraints. In addition, data acquisition and the method of growth instigation are two specific problems being examined, as they will be encountered in interfacing with the GAS project. Flight testing will be two-fold, measurement of algae growth in micro-g and separation of algae from growth medium in an artificial gravitation field. Post flight results will provide information on algae viability in a micro-g environment as reflected by algal growth rates in space. Other post flight results will provide a basis for evaluating techniques for harvesting algae. The results from the GAS project will greatly assist the continuing effort of developing the CELSS and its applications for space.

ONCE UPON A TIME, IN A GALAXY FAR, FAR AWAY

Single cell plant growth in a micro-gravity environment may become crucial for the eventual long-term human habitation of remote space. Currently large fractions of all payloads used in manned space efforts are used to carry water, oxygen, and food into space. In the future, continually replenishing the supplies of a space station will not be economically feasible because of the length of planned missions and the distances from Earth such missions will involve. For example, the minimum cost to transfer a kilogram(kg) of supplies to geosynchronous orbit is approximately 28,600 dollars. For a ten man crew approximately 7,700 kgs of food and water would need to be supplied every six months. This results in a net cost of 220,000,000 dollars every six months just to replenish the crew's food and water supply. A comparison of such an "open" food system with a 97% "closed" supply system as might be made available through a CELSS indicates that for a crew of twelve, 455,000,000 dollars would be saved over a fifteen year period. For such economic reasons, research is currently being performed in developing a Controlled, or Closed, Ecological Life Support System (CELSS), as an alternative to resupply. It also is clear that very remote missions must depend on regenerative life support because resupply would be almost impossible to achieve in a safe reliable fashion. The CELSS is a bioregenerative system, that supplies food for the crew, recycles oxygen, water, and wastes, and maintains itself as a biologically viable entity. This system is considerably more cost beneficial than a system involving resupply, and is therefore considered crucial to supporting a variety of proposed manned space efforts.

Algae has the characteristics to make it the prime candidate as an integral part of a CELSS. Algae requires low maintenance and small volumes. In addition, it has a high ratio of useful biomass (protein and carbohydrate) to nonuseful biomass and provides necessary conversion of carbon dioxide to oxygen. Energy for these bioconversions can be made directly available from the sun. Limited considerations of the behavior of algae in micro-gravity have been completed. Research of particular interest was performed by Steven Walker of Utah State University, 1980. This research however, deals only with the growth of algae. *Chlorella*, in a micro-gravity environment and not harvesting. The present paper presents work in progress at the University of Colorado Aerospace Engineering Sciences Department in developing a Get Away Special (GAS) experiment designed to determine the utility of a specific method 1) to ensure rapid algae growth and 2) to achieve effective algae harvesting in a micro-g environment.

The proposed experiment involves two stages. The first stage involves controlled time periods for algal growth both with and without artificial gravity. On orbit data acquisition will be accomplished using pH indicators and turbidity measurements of growth documented photographically through appropriately positioned fiber optic sensing elements. The growth stage will provide information about the viability of lower plants, specifically algae in a space environment where the separation of fluid and gases is absent. The question arises as to whether the oxygen and carbon dioxide behavior will aid or inhibit algae growth. The second stage is a fluids management experiment involving multi-phase medium separations. The three phases to be separated are gases, nutrient solution, and algae. The data received from this part of the experiment will aid in designing a fluids management system with applications for supporting fluid transfers in space, fluid venting and gauging in space as well as a variety of space habitation needs. The main areas of research for this experiment deal with the choice of algae, maintenance of a suitable growth atmosphere, data acquisition, algae storage, growth instigation in orbit, and actual harvesting of algae.

DECISIONS, DECISIONS

In order to conduct both the ground and space facets of a successful experiment several important and limiting constraints must be met. The main experimental related to space tests

is time. There are two major difficulties encountered in observing the time guidelines. An approximate three month storage phase occurs while the canister is waiting for lift-off. To assure that the necessary growth rate is obtained during the experiment, no algae growth can occur prior to instigation on orbit. Once on orbit instigation occurs, two hours has been allotted to the complete fluids management experiment, largely because of power limitations. This creates the need for a fast growing strain of algae. Other constraints include the need for correct spectrum lights (twenty-two watts cool white fluorescent) that do not alter the test chamber ambient temperature from 40 ± 2 degrees Celsius. The constraints imposed by the CU Get Away Special project for power and space are 112 watt-hours and approximately ten liters devoted to the total fluids experiment. NASA's safety standards require that the algae and associated media be non-toxic. Each of these constraints required considerable ground testing before an appropriate flight configuration could be finalized.

The first step in ground testing was to identify three strains of algae which appeared to satisfy the constraints of a CELSS. These strains are *Anacystis nidulans*, *Chlorella*, and *Euglena gracilis*. Laboratory testing was done to determine growth rates under a variety of light and nutrient conditions. The pigmentation increments of the growing algae was noted since this would be a critical element in photographically recording growth rates. (See Table I) The testing yielded data that corroborated available data that indicates *Anacystis nidulans* has the fastest growth rate and the best pigmentation for the data acquisition phase of this GAS experiment. Research indicates that *Anacystis nidulans* satisfies the NASA constraint that it is non-toxic. (Gorham, 1962)

Table I

Growth Characteristics for Flight Simulations					
algae	class	maximum growth rate (k)	simulation growth rate (k)	optimal growth temperature (C)	pigmentation and sedimentation
Anacystis nidulans	blue-green	3.55 ⁽¹⁾	0.98	41° C	vibrant blue-green color no sedimentation
Chlorella	green	0.56 ⁽²⁾	0.15	25° C	light green sedimentation
Euglena Gracilis	euglenoid	—	—	—	no visible growth no further research

(1) reference Kratz and Myers, 1955

(2) reference Fogg, 1965

After selecting *Anacystis nidulans* as the test algae, more detailed experiments were done assessing altered growth media, carbon sources, heat and light. These variables were evaluated with regard to growth rates and latencies to achieve maximal growth rates. Five different growth media were researched, four of which were mixed in the laboratory, and one of which was purchased premixed. The premixed medium, Alga-Gro Freshwater medium, along with Kratz and Myers medium "C", and Popcock's medium all supported robust growth. The other two mediums, Grand Island Biological cell culture medium, and Beijerinck's medium did not support adequate growth in the laboratory test conditions. Currently, Alga-Gro and Kratz and Myers medium "C" are the two media being considered for use during flight testing. Kratz and Myers medium "C" is slightly favored since, unlike Alga-Gro, the exact chemical composition is known and can easily be altered to fit any changing or unanticipated needs of the experiment. (See Table II)

Table II
Medium C

salt	concentration, g/l
MgSO ₄ ·7H ₂ O	0.25
K ₂ HPO ₄	1.00
Ca(NO ₃) ₂ ·4H ₂ O	0.025
KNO ₃	1.00
Na Citrate 2H ₂ O	0.165
Fe ₂ (SO ₄) ₃ ·6H ₂ O	0.004
A ₅ Microelements ^a	1.0ml

^a A₅Microelements stock solution : H₃BO₃, 2.86g/l; MnCl₂·4H₂O, 1.81g/l; ZnSO₄·H₂O, 0.222g/l; MoO₃(85%), 0.0177g/l; CuSO₄·5H₂O, 0.079g/l

Growth Medium
Adapted from Kratz and Myers, 1955

Not all of the growth medium recipes contained a separate enriched source of carbon that is a necessity for the rapid growth of blue-green algae. Several methods of supplementing a growth medium lacking a carbon source were examined. One method was to add glucose, C₆H₁₂O₆, to the mediums that did not contain any separate carbon source. This method did not support growth. Glucose, a six-sided sugar, is apparently too large to diffuse across the algal membrane and algae are primitive cells that do not have a glucose transport mechanism. The second attempt was to add biotin, C₁₀H₁₆O₃N₂S to the medium. Biotin is a colorless crystalline growth vitamin of the vitamin B complex. This method did not result in any recordable growth by the algae either. The final method, suggested in an article by Kratz and Myers 1955, was to bubble CO₂ through the medium. Limited bubbling was done on their medium "C". The resulting medium supported growth well; it has a growth rate comparable to Algal-Gro under the same heat-light conditions. The bubbling of the CO₂ was accomplished by containing dry ice in a stopped flask with a tube connecting it to a flask of growth medium, for approximately five minutes. To achieve maximum growth rates for *Anacystis nidulans*, constant bubbling of air containing 4% CO₂ through the growth chamber is recommended by Kratz and Myers. Constant bubbling is not feasible for implementation in this experiment due to volume and power constraints. Rather, the growth medium would have to be saturated with carbon dioxide prior to lift off and the algae would be inoculated into the growth medium while on orbit.

The optimal temperature for growth of *Anacystis nidulans* is 41 degrees Celsius. For test simulation purposes, *Anacystis nidulans* was grown at room temperature and in an incubator set at 40 degrees Celsius. Rate of growth can be expressed in terms of a specific growth rate, k , defined by the growth equation $\log_{10} N/N_0 = kt$. The variable k is expressed in log₁₀ units per day: when $k = 0.301$ the population doubling time is one day. For *Anacystis nidulans*, Kratz and Myers report $k = 0.87$ for growth at 25 degrees Celsius and $k = 3.55$ at 41 degrees Celsius. Tests with simulated GAS experiment conditions, using *Anacystis nidulans* as the test algae, resulted in $k = 0.120$ to $k = 0.181$ at a growth temperature of 25 degrees Celsius and $k = 0.380$ to $k = 0.903$ at a temperature of 40 degrees Celsius. Kratz and Myers used optimal growth conditions, while testing at the University of Colorado's biolab was conducted under conditions similar to flight. During flight, a thermal blanket will be wrapped around the storage container in which the algae growth will occur. This insulation coupled with lighting control should permit some degree of temperature regulation during on orbit algae growth. The thermal blanket and light controls are expected to help maintain a constant temperature of 40 ± 2 degrees Celsius.

The sensitivity of the algae to light levels was also considered. No growth is recorded when the algae does not receive any light. Cool white fluorescent light is recommended by the Carolina Biological laboratories because of the spectrum of light emitted. Accordingly, twenty-two watt cool white fluorescent lighting was used in the laboratory and was found to sustain rapid algae growth. During flight, such a twenty-two watt cool white fluorescent light tube will be used adjacent to the growth chamber to provide the light necessary for growth. As noted above, lighting cycles may have to be modified to support appropriate temperatures in the growth chamber.

BEHIND THE SCENES; THE DIRTY WORK

Growth rates were obtained by taking population measurements over time. These growth measurements were collected using a drying and weighing system. To acquire a data point, 100 milliliters(mls) of algae in its growth solution were spun down in 15 ml test tubes in a centrifuge at roughly 1300g. The supernatant was taken out of the tubes leaving an algae rich solution. The algae rich solution was then aspirated through 0.22 micron Millipore filters. The Millipore filters were previously weighed and saturated with 0.15 M NaCl solution to prime them for the filtration process. The algae solution was aspirated two times through the filter. The filter was then placed on a glass dish for twenty hours in an oven set at 50 degrees Celsius. After the drying time had elapsed, the filter was then reweighed. This is a simple effective way of determining the dry weight of a sample of algae. And, from such dry weights the growth rate of the algae can be determined. (See Figure III)

Since the above procedure does not lend itself to on orbit measures of algae growth, alternative schemes were assessed. The pH changes associated with growth were a particularly attractive alternative since measurements could be recorded in a number of different ways. For testing, bromthymol blue and phenol red were the two chemical pH indicators that were added to the algae solutions. Bromthymol blue has a pH range of 6.0 to 7.6, whereas phenol red has a range of 6.8 to 8.4. The color ranges were from yellow to deep blue or to dark red, respectively. To establish the utility of the two pH indicators, a ten ml sample of active algae growth solution containing the indicator was collected daily. The samples were stored in a refrigerator in the dark. Photos were then taken to provide visual indications of growth that can be later calibrated. This also tested the algae's viability when mixed with an indicator. During actual flight, an indicator with a large color range in a small pH change will be used, as the growth time is restricted to two hours. A change in pH indicates a change in acidity associated with growth and utilization of the carbon dioxide. The formula that relates pH to H^+ ions is $[H^+] = 10^{-(pH)}$. A change in pH of an algal solution indicates growth. (See Figure IV)

WHEN SHE FLIES

As stated previously, the time constraints of the GAS Experiment present a unique problem. The algae will be stored for the approximate three months prior to launch with neither heat nor light. During this phase, no growth may occur. Lyophilization, freeze drying, was one option considered for storage. It was rejected due to an accompanying lag time needed before growth occurred. Another approach considered is storing the algae in a medium with vital growth components missing. The question arises as to whether or not a medium that is deficient in vital ingredients will support life for three months. A method of storage being examined currently is storage of the algae in a medium that brings the algae to asymptotic growth, or in a stationary phase. Algal solutions that are asymptotic are being subjected to several months of tests in an environment lacking illumination or heat. Research has been performed to find the algae concentrations at which the growth rate is asymptotic. This information coupled with the mode of storage will determine an optimal initial population for the experiment.

Once the on flight experiment begins, rapid growth of the algae is imperative. Little growth lag time may occur during the two hours of data acquisition. It is envisioned that instigation of algae growth will be accomplished using a piston action to puncture plastic films which will allow the mixing of two separate mediums. One medium will contain the asymptotic algae, the other will contain the rapid growth instigating nutrients. Data acquisition will then occur using pH indicators, fiber optics, and photographs taken at specific time intervals.

The second stage of the experiment involves separation of the algae from gasses and growth medium. This will provide useful information for recovering solids suspended in fluids, specifically, algae harvesting. Separation will be achieved through centrifugation and accompanying fluid density gradients. A clumping agent may be added to aid in density stratification of the three phases. Data acquisition during this stage will also be accomplished using time interval photography.

APODOSIS

The GAS Project is limited; the strict constraints do not allow an ideal testing situation and there are many potential failure points. Loss of power, component breakage during launch, misalignment of data acquisition equipment, or loss of thermal control, would all be catastrophically detrimental to experiment success. In most operational scenarios, even those including such a detrimental event, valuable data will be generated. For example, if camera failure occurs, the examination of the algae remaining in the centrifuge, storage container, and connecting tubing, will reveal minimal data on growth. In the case of a total or partial loss of thermal control, the growth of *Anacystis nidulans* will be diminished, possibly below the level of recordable growth. Lighting failure would be more catastrophic than thermal failure. Ground testing indicates that very minimal growth occurs in situation with no illumination. In addition, visual methods of data acquisition of growth and separation would be rendered useless. Except in the extreme cases, however, these failures would only limit and minimize, not eliminate useful data.

Experiment failures could also occur due to testing in a micro-g environment. The possible reluctance of gases (CO_2 , O_2) to separate from the medium would affect the pH, and in turn make the calibration of data difficult. Ground testing will provide information about latencies to growth maxima. This information may become irrelevant in a micro-g growth environment, as latencies to growth maxima may change. These events should not be viewed as providing useless information, as important data about the growth of algae in a micro-g environment will still be retrieved.

The CU GAS experiment is expected to fly as soon as the Shuttle Program becomes operational. Decisions have been made concerning the algae, *Anacystis nidulans* grown with a temperature of 40°C and twenty-two watts cool white fluorescent lighting. On orbit data acquisition will be accomplished using pH indicators and fiber optics. Much research still needs to be performed in order to make the GAS project flight ready. Future testing will include; triggering rapid growth of algal solutions currently being stored in a stationary growth phase, continued pH calibration for data acquisition, experimentation in promoting faster algal growth, and centrifugation at speeds comparable to speeds encountered during in-flight experimentation to examine growth rates and separation. Future, as well as completed ground testing not only aids in the preparation of the CU GAS Project, but also provides valuable background information for the future development of a CELSS.

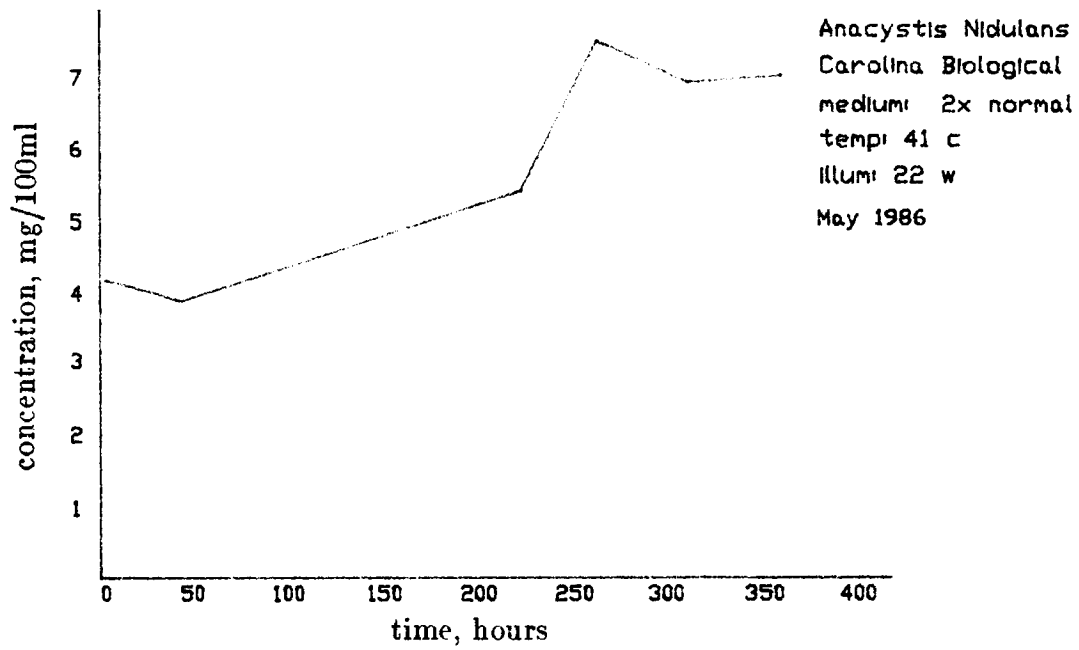
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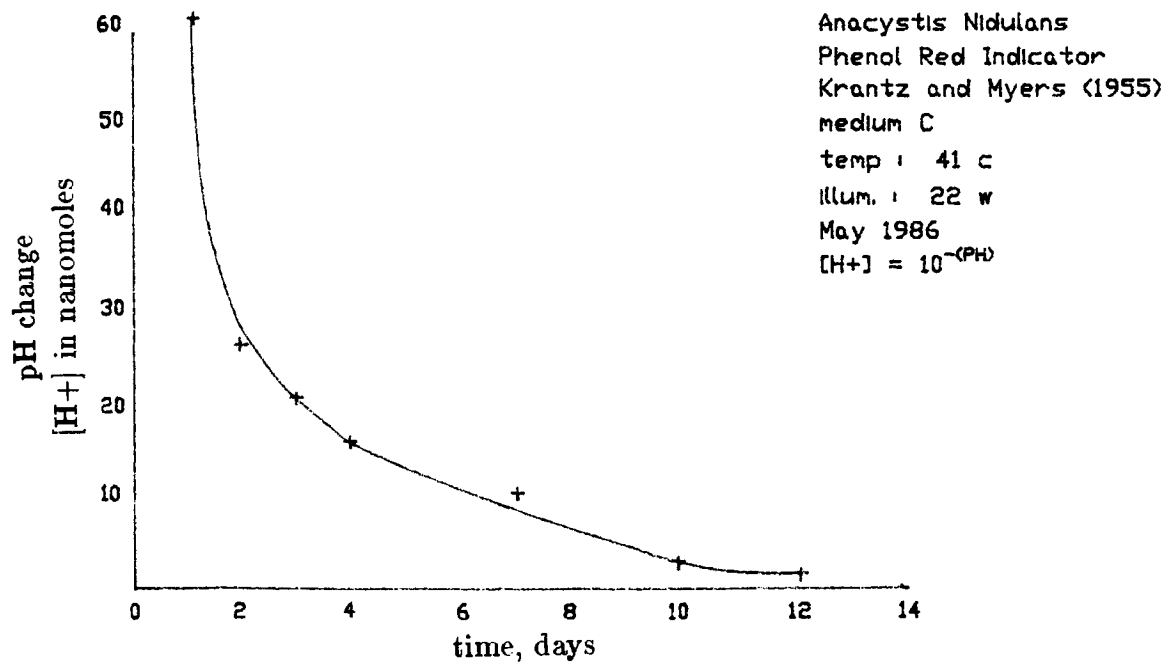
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Figure III



Growth Curve

Figure IV



pH Change with Days of Growth